IMMUNOGENIC COMPOSITIONS FOR INDUCTION OF ANTI-TUMOR IMMUNITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a division of U.S. application 09/445,602, which was filed on March 23, 2000; which is the national stage under 35 U.S.C. §371 of PCT/IL98/00211, filed June 9, 1998, the entire contents of each being incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to the use of an immunogenic composition for the induction of anti-tumor immunity in mammals, said composition comprising a pharmaceutically acceptable carrier and an immunogen selected from the group consisting of: (i) a monoclonal antibody (mAb) to the tumor-associated antigen (TAA) p53, (ii) a fragment of an anti-p53 mAb, (iii) a peptide based on a complementarity-determining region (CDR) of an anti-p53 mAb, which peptide is capable of eliciting antibodies to p53, and (iv) a nucleic acid sequence coding for the variable region of an anti-p53 mAb, and to a method for induction of anti-tumor immunity in a subject comprising administration of said immunogen.

ABBREVIATIONS

[0003] The following abbreviations will be used herein in the specification and claims: **anti-id** - anti-idiotype; **mAb** -

monoclonal antibody; **TAA** - tumor-associated antigen; **CDR** - complementarity-determining region of the heavy or light chain of a m-Ab.

BACKGROUND OF THE INVENTION

5   [0004] The rejection of transplanted cells and tissues of allogeneic origin proves that the immune system is capable of destroying its targets. It has long been a goal of immunologists to direct the destructive capability of the immune system against a person's tumor cells and thereby

10   effect the rejection of the tumor and the cure of the patient. Immunotherapy would be especially useful to rid a person of residual tumor cells that have spread beyond the site of the primary tumor mass. The primary tumor can usually be resected surgically or irradiated by local radiotherapy. The patient

15   too often succumbs later, however, to metastatic tumor cells that have spread to other parts of the body. Immunotherapy would be an ideal way to destroy nests of metastatic tumor cells before they grow into large fife-threatening tumors. Lymphocytes patrol the tissues and lymphocytes sensitized to

20   the tumor cells could kill the metastatic tumor cells remaining after resection of the primary tumor mass.

[0005] The problem for immunotherapy, however, is to activate the immune system against antigens that can mark the tumor cells for destruction. It had been hoped that tumors

might bear "foreign transplantation" antigens produced by viruses or created by immunologically significant mutations of genes in the tumor cell. It now turns out that such tumor specific transplantation antigens (TSTA) are rare.

5 [0006] Tumor cells are characterized immunologically for the most part by TAA, which are molecules that are expressed in normal cells too. These TAA may appear normally during the early development of healthy cells, or they may be expressed normally at lower concentrations than in tumor cells. TAA may
10 also feature minor mutations that do not appear "foreign" to the immune system and thus do not stimulate strong immune responses. TAA, for the most part, are self-antigens and, as such, they are not very immunogenic. The immune system is normally tolerant to the body's own antigens. Therefore, the
15 induction of effective immunity against TAA is tantamount to inducing an autoimmune reaction. Immunotherapy of tumors requires the activation of the equivalent of an autoimmune reaction against the tumor cells. Moreover, it is most desirable to have the autoimmune reaction limited to the tumor
20 itself, so the autoimmune reaction terminates once the tumor cells are destroyed. Nevertheless, autoimmunity can be compatible with life, while metastatic cancer can kill. Therefore, residued autoimmunity is a tolerable price to pay for successful tumor immunotherapy.

[0007] Examples of known TAA include p53 protein, neu differentiation factor (NDF), epidermal growth factor (EGF), carcinoembryonic antigen (CEA), and tyrosinase enzyme.

[0008] The p53 protein is the product of a tumor suppressor gene that functions to arrest the growth of mutated or aberrant cells. The p53 protein is a transcription factor that binds specifically to a consensus site present in the regulatory sequences of p53-dependent genes (el-Deiry et al., 1992; Zambetti and Levine, 1993). Mutation of the p53 gene in the domain encoding binding to the specific DNA regulatory site causes a loss of tumor suppression (Zambetti and Levine, 1993). Therefore it is not surprising that a significant proportion of natural human tumors bear mutated p53 (Hollstein et al., 1991). For reasons that are-not entirely clear, tumor cells also appear to accumulate wild-type p53 and not only mutated p53 in their cytoplasm (Moll et al., 1995). Thus the wild-type p53 molecule, and not only the mutated p53 molecule, can serve as a target for a potentially therapeutic anti-tumor immune response.

[0009] Inactivation of the p53 tumor suppressor protein by mutation of the gene or by viral insertion, gene rearrangement, or other causes is a common event in human cancers. Point mutation or deletion of the p53 gene is the most common genetic aberration in human neoplasms.

Approximately 70% of colon cancers, 30 to 50% of breast cancers, 50% of lung cancers, and almost 100% of small-cell carcinomas of the lung harbor p53 mutations (Hollstein et al., 1991). The development of a tumor is often associated with accumulation in the cancer cells of the p53 protein, wild-type or mutant. Furthermore, mutated p53 proteins are tumor-specific antigens that can be recognized as targets by the immune system (Melief and Kast, 1991; Yanuck et al., 1993). Cancer patients can manifest immune responses directed to wild-type and mutant p53 proteins. The p53 protein, mutant and wild-type, can accumulate in the cytoplasm of cancer cells, and cancer patients have indeed been found to produce antibody (Lubin et al., 1993; Schlichtholz et al., 1992) and T cell responses to p53 (Houbiers et al., 1993; Tilkin et al., 1995). Normal cells express p53 to a much lower degree and, unlike tumor cells, normal cells show no accumulation of p53 in the cytoplasm. Thus, tumor cells and normal cells differ in both the amount and compartment of p53 expression. For these reasons, the wild-type p53 molecule, and not only the mutated p53 molecule, can serve as a target for a potentially therapeutic anti-tumor immune response.

[0010] To identify T-cell epitopes in p53, Houbiers et al., 1993, synthesized peptides of wild-type p53 and peptides with the point mutations of p53 detected in colorectal and ovarian

cancers. Some of the p53 peptides were shown to bind in vitro to HLA-A2.1 molecules and to induce specifically cytotoxic T lymphocytes (CTL) clones. Characterization of anti-p53 immunity and its implications for tumor therapy have been

5 studied using peptides derived from wild-type or mutated p53 sequences to elicit CTL responses in experimental animals (Noguchi et al., 1994; Noguchi et al., 1995; Yanuck et al., 1993). Mouse fibroblasts transfected with a mutated human p53 gene were specifically killed by CD8^{+} CTL from the spleens of

10 mice that had been pulsed with a 21-amino acid peptide encompassing a p53 point mutation from a human lung carcinoma (Yanuck et al., 1993). A nonapeptide containing a codon 234 mutation (234CM) induced CD8^{+} CTL that lysed a 234CM-pulsed PIHTR mastocytoma cell line (Noguchi et al., 1994). Mice

15 immunized with peptide 234CM were resistant to challenge with Meth A sarcoma cells (Noguchi et al., 1994), and vaccines containing peptide 234CM in the QS-21 adjuvant caused regression of established Meth A tumors in mice treated with IL-12 (Noguchi et al., 1995).

20 [0011] Thus, both mutated p53 and wild-type p53 are tumor-associated antigens and attempts have been made to use these molecules as immunogens for tumor immunotherapy (Houbiers et al., 1993; Noguchi et al., 1994; Noguchi et al., 1995; Yanuck et al., 1993; published PCT Application WO 94/02167).

However, p53 is not very immunogenic, probably because it is a
self-protein and therefore immunologically tolerated.

[0012] An antibody binds to an antigen at its variable
region (antigen-binding site). Therefore, the variable
regions of antibodies have three-dimensional structures that
are complementary to the structures of the antigenic
determinants the antibodies recognize.

[0013] The binding site of the antibody complementary to
the structure of the antigen is created by hypervariable
regions of the light and heavy chains of the Fab portion of
the antibody. These binding site structures are formed by the
collective aggregate of the CDR of the light and heavy chains
of the immunoglobulin molecule (Alzari et al., 1988).
However, an antibody itself, when recognized by another
antibody, can be considered to be an antigen. In the case
where structures of the variable regions of the antibody are
recognized, these structures are called idiotypes Gd), and the
antibodies that recognize the idiotypes of the antibody are
called anti-idiotypic (anti-id) antibodies. The structure
corresponding to the antigenic determinant of the antibody is
called an idiotope (Jerne, 1974).

[0014] It has been reported that immunization with mAbs can
induce immune responses that extend beyond the specificity of
the antibody (Takemori et al., 1982), probably by anti-

idiotypic connectivity (Jerne, 1974; Cohen, 1989, 1992) based

on idiotypic determinants in the variable (V) region of the

immunizing mAb (Bruggemann et al., 1980). According to

idiotypic antibody network terminology, Ab1 is the first

antibody, the antibody binding to the antigen, and Ab2 is the

anti-idiotypic antibody to Ab1. The variable region of Ab2

may mimic the conformation of the antigen because both the

antigen and Ab2 can be bound by Ab1. Ab3 is the anti-

idiotypic antibody to Ab2. Because of the chain of structural

complementarity, Ab1 and Ab3 can have similar specificity for

the original antigen.

[0015] Antibodies have been used in the past in tumor

immunotherapy in two ways: Ab1 antibodies as tumor-specific

antigens on B lymphoma cells, and Ab2 antibodies as anti-

idiotypic mimics of tumor antigens. Ab1 idiotypic determinants

expressed by immuno globulins on the surface of neoplastic B

cells have been used in experimental models as tumor-

associated targets to induce protective immunity (Ab2) against

B cell lymphomas which, unlike solid tumors, are particularly

sensitive to antibodies (reviewed by Yefenof et al., 1993).

However, Ab1 idiotypic determinants are unique to each B-cell

tumor, and the practical requirements of preparing an

individual protein vaccine for each patient has made the

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application to the clinic difficult and expensive (Stevenson et al., 1995).

[0016] Ab2 antibodies mimicking TAAs of various kinds have been used to induce antibodies (Ab3) to tumor antigens (reviewed by Wettendorf et al., 1990). However, Ab2 immunization has been usually less successful than has immunization with the TAAs themselves, (Wettendorf et al., 1990).

[0017] With regard to the possible anti-cancer effects of anti-p53) antibodies, cancer patients have been found to produce antibodies to the amino terminus of the p53 molecule, but these antibodies appear to mark the development of cancer rather than to protect against the disease (Soussi, 1996). Investigation of the effects of immunity to the central and carboxy domains of p53 might therefore be of some importance.

[0018] Published International PCT Application No. WO 94/12202 describes the activation of a mutant p53 that occurs at elevated levels in tumors and does not substantially suppress tumor growth, for specific DNA binding, wherein the mutant p53 is activated with a ligand capable of binding to, and activating the mutant p53), wherein the ligand may be the anti-p53 mAb 421 which binds to the carboxy terminal region of p53, or the bacterial heat shock protein DnaK, or a ligand which binds effectively to the same site on the mutant p53.

SUMMARY OF THE INVENTION

[0019] It is an object of the present invention to provide a novel way of inducing effective anti-tumor immunity without apparent harm to the patient.

[0020] It has now been found in accordance with the present invention that immunization of BALB/c or C57BL/6 mice with anti-p53 mAbs (Ab1) directed to different domains of the p53 molecule induces spontaneously antibodies to p53 (Ab3), and that anti-DNA antibodies (Ab2) are generated if Ab1 is directed to a DNA-binding domain of p53.

[0021] The present invention thus relates to the use of an immunogen selected from the group consisting of:

(i)   an anti-p53 mAb;

(ii)   a fragment of an anti-p53 mAb;

(iii) a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb, which peptide is capable of eliciting antibodies to p53; and

(iv)   a DNA molecule coding for the variable (V) region of an anti-p53 mAb in a suitable gene delivery vehicle,

for the preparation of a pharmaceutical composition useful for induction of anti-tumor immunity in mammals, for activating an enhanced immune response to a p53 molecule in mammals, and/or

for induction of immune responses to mutated and wild-type forms of p53 in mammals.

[0022] In another aspect, the present invention relates to methods for induction of anti-tumor immunity in a mammal, for activating an enhanced immune response to p53 in a mammal, and/or for induction of immune responses to mutated and wild-type forms of p53 in a mammal, which comprises administering to said mammal an effective amount of an immunogen selected from the group consisting of:

(i)   an anti-p53 mAb;

(ii)   a fragment of an anti-p53 mAb;

(iii)   a peptide based on a CDR of the heavy or light chain of an anti-p53 mAb, which peptide is capable of eliciting antibodies to p53; and

(iv)   a DNA molecule coding for the variable (V) region of an anti-p53 mAb in a suitable gene delivery vehicle.

[0023] The anti-p53 mAb used according to the invention may be directed against different domains of wild type p53 or a mutant p53, and may be a murine, human or humanized anti-wild type or anti-mutant p53 mAb.

[0024] The anti-p53 mAb fragments that can be used according to the invention include antigen-binding fragments (Fab), F(ab')2 or any other type of antibody molecule,

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including single chain Fv fragments of antibodies, as long as

such antibody fragments are able to bind p53 as well as

peptides based on a CDR of the heavy or light chain of an

anti-p53 mAb, which peptides are capable of eliciting

5   antibodies to the p53 without necessarily binding p53.

[0025] In still a further aspect, the present invention

relates to a method for the generation of sequence-specific

anti-DNA antibodies which comprises immunizing a mammal with a

mAb directed to a domain containing a DNA-binding site of a

10   DNA-binding protein, e.g. p53, and recovering the thus

elicited sequence-specific anti-DNA antibodies.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Fig. 1 shows the retardation in gel of the p53-

specific oligonucleotide sequence by anti-DNA antibodies

15   developed in mice immunized with anti-p53 mAb 246, but not

with mAb 240, mAb 421, mAb 9E 10 and mAb R73.

[0027] Figs. 2A-2B show immunoprecipitation of labeled p53

produced by reaction of an extract of Meth A tumor cells,

metabolically labeled with 35S-methionine, with anti-p53 mAbs

20   421, 246 and 240 (Fig. 2A) or with antisera induced by

immunization with mAbs 421, 240 (Fig. 2A), or mAb 246 and

control 9E 10 (Fig. 2B).

[0028] Fig. 3 shows the sequences (SEQ ID NO:1 to SEQ ID

NO:8)of the heavy (VH) and light (VL) chains of the anti-p53

mAbs 240, 246, 248 and 421, in which the CDR sequences are underlined.

[0029] Fig. 4 shows the protective effect of immunizing mice with the anti-p53 mAbs 240, 246 and 421 against the development of lung carcinoma metastasis in mice. mAb R73 was used as a control immunogen. Dots indicate the weight of individual lungs, bars the median of each group. Normal lung weight is around 200 mg.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The present invention relates, in one aspect, to the use of an Ab1 anti-p53 mAb to generate an Ab3 anti-p53 response. Presumably, the chain reaction from Ab1 to Ab3 implies the generation of Ab2 antibodies as intermediates.

[0031] To activate an enhanced immune response to p53 according to the invention, an anti-idiotypic; network (reviewed by Nisonoff, 1991) inducible by immunization with antibodies as immunogens rather than with antigens, was exploited.

[0032] The p53 molecule has two attributes of immunological interest: (a) because p53 binds DNA, immunity to p53 may lead to anti-DNA antibodies by an anti-id network: antibodies to a DNA-binding site of p53 can mimic DNA and, therefore, such anti-p53 antibodies might induce anti-DNA antibodies as anti-

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ids; and (b) because p53 accumulates in transformed cells, immunity to p53 may have an anti-tumor effect.

[0033] The generation of antibodies to DNA has been difficult because the DNA molecule is poorly immunogenic. In particular, it would be desirable to obtain antibodies to specific DNA sequences as such antibodies can be used to detect the presence of such sequences for purposes of diagnosing whether an individual has a specific gene or promoter sequence. A specific antibody would have an advantage over currently used PCR (polymerase chain reaction) techniques, because antibody binding, unlike the PCR, is easily quantifiable and needs no primers or enzymatic replication. In particular, sequence-specific anti-DNA antibodies can be used in diagnostics, for example, in detecting critical sequences in the breeding of animals and plants, in the identification of bacteria and other parasites, in determination of paternity and maternity, in forensic medicine. Specific anti-DNA antibodies also can be useful in the isolation of specific genes for DNA vaccination, gene cloning, and gene sequencing. Antibodies to specific sequences of DNA might also have a use in the activation or inhibition of particular genes for therapeutic purposes in plants, animals or humans. It has been shown that antibodies penetrate into living cells (Alarcon-Segovia et al., 1996;

Madaio et al., 1996; Yanase et al., 1994; Ma et al., 1991) and anti-DNA antibodies might be able to exert effects within living cells. But DNA in general, and certainly specific sequences of mammalian DNA, are not immunogenic.

5   [0034] According to the present invention, the p53 molecule was used as model of a DNA-binding protein in order to learn whether sequence-specific anti-DNA antibodies might be generated by an anti-id network centered around immunity to a DNA-binding protein. The p53 molecule might serve as a model of a well-characterized DNA-binding protein that recognizes a highly specific p53 regulatory site (el-Deiry et al., 1992). This domain makes it possible for the p53 molecule to bind to the p53-specific DNA regulatory site, leading to growth arrest of the aberrant cell. Antibodies to the specific DNA-binding,

15   site of p53 can be used to determine whether specific anti-DNA antibodies (as Ab2) can be generated, by way of an anti-id network, using antibodies to the specific DNA-binding domain of p53 (as Ab1). Because of the structural similarity between DNA and RNA, it is obvious that the specific anti-DNA

20   antibodies could also recognize specific RNA.

[0035] Induction of an immune response to specific DNA (Ab2) and to mutated and wild-type forms of p53 (Ab3) is provided according to the invention by immunization with mAbs (Ab1) specific for particular domains of wild-type or mutated

p53 proteins. The induction of anti-p53 antibodies (Ab3) is

hereon shown to be associated with the development of

resistance to challenge with a murine fibrosarcoma tumor Meth

A and with inhibition of lung metastases produced by the

5   spontaneously metastazing 3LL tumor. Since over-expression

and/or accumulation of p53 antigen is common to many tumors,

immunization with antibodies directed to the p53 protein, by

eliciting an idiotypic network response, may be useful in the

development of a general therapy for different cancers,

10   particularly since effector T cells are activated in such

networks.

[0036] The induction of specific anti-DNA antibodies by

immunization with a mAb to the domain of p53 that binds

specific DNA indicates that anti-DNA antibodies can indeed

15   arise by an anti-id network. Thus, the anti-id network

appears to preserve structural similarity with particular p53

domains.

[0037] Thus, in particular embodiments of the present

invention, immunization either with an anti-mutant p53 mAb

20   such as mAb 240 that is specific for a mutated p53)

conformation (Gannon et al., 1990), or with an anti-wild type

p53 mAb such as mAb 246 that is specific for the native

conformation of p53, at the edge of the DNA-binding site of

native p53 (Yewdell et al., 1986), or with a mAb against both

the wild-type and mutant p53, such as mAb 421 that is specific

for the carboxy terminal domain of p53 conserved in both wild-

type and mutant p53 (Arai et al., 1986), induced anti-p53

antibody titers both in BALB/c and in C57BL/6mice, apparently

by way of an anti-idiotypic network. This anti-p53 immunity

was associated with the acquisition of the ability to reject

both primary tumors and metastatic tumors.

[0038] However, the development of specific anti-DNA

antibodies (Ab2) varied with the domain of p53 recognized by

the Ab1 used to activate the network. Both the mAb 240

specific for mutated p53, and the mAb 421 specific for the

carboxy terminus, did not induce sequence-specific anti-DNA

antibodies. However, the mAb 246 induced antibodies to the

p53-specific DNA sequence. Thus, sequence-specific anti-DNA

antibodies can be produced by immunization with anti-p53 mAbs

specific to the central DNA-binding domain of p53, such as mAb

246; and generation of anti-DNA antibodies can be avoided by

the use of an anti-P53 antibody, such as mAb 240, that does

not bind a domain of p53 that binds to DNA.

[0039] The present invention encompasses the use of any

anti-p53 mAb, or a fragment thereof Since different mutations

of p53 may generate a common mutant conformation (Gannon et

al. 1990), it may not be necessary to tailor a mAb to each

mutation of p53. Thus, according to the present invention,

anti-p53 mAbs to either native or mutant p53 can serve as immunotherapeutic agents of wide applicability in the treatment of cancer. The therapeutic anti-p53 mAbs can be of various kinds, including murine, human, or "humanized" mouse antibodies, all of them isolated or prepared in different ways by standard procedures.

[0040] Anti-p53 mAb fragments according to the invention include antigen-binding fragments (Fab), F(ab')2 or any other type of antibody molecule, including single chain Fv fragments of antibodies, as long as such antibody fragments are able to bind to p53.

[0041] The use of genetically engineered humanized mAb or of mAb fragments may be preferred according to the invention because they minimize the amount of foreign protein to be injected and avoid an immune response that may be generated against a murine mAb.

[0042] The structural correlates of idiotopes that define the unique binding specificities of antibodies include the CDR hypervariable segments of the immunoglobulin heavy andlight chains (Alzari et al., 1989). Thus, according to the present invention, synthetic peptides containing the sequence of a CDR of the heavy or light chain of an anti-p53 mAb, and not only the intact mAb molecule, induce anti-p53 immunity by the anti-idiotypic network.

[0043] The invention thus further relates to synthetic peptides based on a CDR of the heavy or light chain of an anti-p53, mAb, and salts and chemical derivatives thereof. Examples of such peptides are peptides containing one or more of the sequences of the CDR2 and CDR3 of the heavy chain and of the CDR3 of the light chain of the mAbs against p53, which sequences are underlined in Fig. 3 as follows:

(i) Peptides, herein designated Ia-Ib, based on the CDR2 and CDR3, respectively, of the heavy chain (240VH), and peptide Ic based on the CDR3 of the light chain (240VL), of the anti-p53 mAb 240, of the sequences:

(Ia)   Glu-Ile-Asp-Pro-Ser-Asp-Ser-Tyr-Thr-

        Asn-Tyr-Asn-Gln-Asn-Phe-Lys-Asp   (SEQ ID NO:9)

(Ib)   Leu-Leu-Arg-Tyr-Phe-Ala-Met-Asp-Tyr   (SEQ ID NO:10)

(Ic)   Gln-His-Ile-Arg-Glu-Leu-Thr-Arg   (SEQ ID NO:11)

(ii) Peptides, herein designated IIa-IIb, based on the CDR2 and CDR3, respectively, of the heavy chain (246VH), and peptide IIc based on the CDR3 of the light chain (246VL), of the anti-p53 mAb 246, of the sequences:

(IIa)   Asp-Ile-Asn-Pro-Asn-Asn-Gly-Tyr-Thr-

        Ile-Tyr-Asn-Gln-Lys-Val-Lys-Gly   (SEQ ID NO:12)

(IIb)   Gly-Gly-Gly-Leu-Lys-Gly-Tyr-Pro-Phe-

        Val-Tyr   (SEQ ID NO:13)

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(IIc)   Gln-Gln-Arg-Ser-Ser-Phe-Pro-Phe-Thr   (SEQ ID NO:14)

(iii) Peptides, herein designated IIIa-IIIb, based on the CDR2 and CDR3, respectively, of the heavy chain (248VH), and peptide IIIc based on the CDR3 of the light chain (248VL), of the anti-p53 mAb 248, of the sequences:

(IIIa) Asp-Ile-Tyr-Pro-Asn-Asn-Gly-Phe-Thr-

Thr-Tyr-Asn-Gln-LysPhe-Lys-Gly   (SEQ ID NO:15)

(IIIb) Ser-Gly-Ser-Arg-Phe-Asp-Tyr   (SEQ ID NO:16)

(IIIc) Gln-Gln-Ser-Asn-Ser-Trp-Pro-Val-His-

Ala   (SEQ ID NO:17)

(iv) Peptides, herein designated IVa-IVb, based on the CDR2 and CDR3, respectively, of the heavy chain (421VH), and peptide IVc based on the CDR3 of the light chain (421VL), of the anti-p53 mAb 421, of the sequences:

(IVa)   Trp-Ile-Asp-Pro-Glu-Asn-Gly-Asp-Thr-

Glu-Tyr-Ala-Pro-Lys-Phe-Gln-Gly   (SEQ ID NO:18)

(IVb)   Tyr-Gly-Asp-Ala-Leu-Asp-Tyr   (SEQ ID NO:19)

(IVc)   Trp-Gln-Gly-Thr-His-Ser-Pro-Leu-Thr (SEQ ID NO:20)

[0044] A "chemical derivative" of a peptide of the present invention, as defined herein, contains additional chemical moieties not normally a part of the peptide. Covalent modifications of the peptides are included within the scope of the invention. Such modifications may be introduced into the

molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Such derivatives include, but are not limited to, esters, N-acyl derivatives, and the like. Many such chemical derivatives and methods of making them are well known in the art.

[0045] Also included in the scope of the invention are salts, both organic and inorganic, of the CDR-based peptides.

[0046] The peptides according to the invention have preferably 9-30 amino acid residues, examples of which are the 17- to 21-mer peptides V, VI, VII, VIII and IX, which are based on the CDR sequences mentioned above as follows: peptide V includes the sequence Ic of the CDR3 of the light chain of mAb 240, peptide VI includes the sequence IVc of the CDR3 of the light chain of mAb 421, peptide VII includes the sequence IIa of the CDR2 of the heavy chain of mAb 246, peptide VIII includes the sequence IIIb of the CDR3 of the heavy chain of mAb 248, and peptide IX includes the sequence IIIc of the CDR3 of the light chain of mAb 248.

[0047] Peptides V to IX have the following sequences:

Peptide V: Tyr-Tyr-Cys-Gln-His-Ile-Arg-Glu-

                Leu-Thr-Arg-Ser-Glu-Gly-Gly-Pro-

                Ser   SEQ ID NO:21

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Peptide VI:      Gly-Val-Tyr-Tyr-Cys-Trp-Gln-Gly-

                 Thr-His-Ser-Pro-Leu-Thr-Phe-Gly-

                 Ala-Gly-Thr-Lys                    SEQ ID NO:22

Peptide VII:     Gly-Asp-Ile-Asn-Pro-Asn-Asn-Gly-

                 Tyr-Thr-Ile-Tyr-Asn-Gln-Lys-Val-

                 Lys-Gly-Lys-Ala                    SEQ ID NO:23

Peptide VIII:    Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Ser-

                 Gly-Ser-Arg-Phe-Asp-Tyr-Trp-Gly-

                 Glu-Gly-Thr-Thr                    SEQ ID NO:24

Peptide IX:      Val-Tyr-Phe-Cys-Gln-Gln-Ser-Asn-

                 Ser-Trp-Pro-Val-His-Ala-Arg-Gly-

                 Gly-Gly-Thr-Lys                    SEQ ID NO:25
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[0048] The present invention also provides a DNA sequence coding for the variable region of a mAb to p53, in a suitable gene delivery vehicle. Any gene delivery vehicle such as viral vectors, e.g. recombinant retroviral vectors and recombinant adenovirus vectors, naked DNA nucleic acid molecules such as plasmids, liposomes, bacterial and eukaryotic cells, as well as other suitable vehicles as described in Vile and Russell, 1994, and in published PCT Application WO 96/21015, both references herein incorporated by reference, can be used according to the invention.

[0049] According to the invention, the immunogens can be used for treatment of primary, as well as metastatic, tumors

of any kind, including but not being limited to brain, breast, ovarian, uterus, skin, lung, esophagus, colon, prostate, kidney and bladder cancers, leukemias and lymphomas.

[0050] A pharmaceutical composition according to the invention comprising an anti-p53 mAb, a fragment thereof or a peptide based on a CDR region of the heavy or light chain of an anti-p53 mAb, together with a pharmaceutically acceptable carrier, can be administered through various routes known in the art, such as oral, intranasal, intravenous, subcutaneous, intramuscular, intraperitoneal, transdermal or other known routes including the enteral route. In a preferred embodiment, the composition comprising the anti-p53 mAb, fragment or CDR-based peptide, is administered subcutaneously.

[0051] When the immunogen is a DNA molecule coding for the variable region of an anti-p53 mAb, it is administered in a suitable gene delivery vehicle as described (Vile and Russel, 1994; WO 96/21015).

[0052] The dosage of the immunogen to be administered will depend on the type of compound used - a mAb, a fragment or a peptide, and upon the age, sex, weight and condition of the recipient. The doses should not be so large as to cause adverse side effects such as unwanted cross-reactions, generalized immunosuppression, anaphylactic reactions and the like.

[0053] It is also encompassed by the present invention to administer to a patient effective amounts of two or more different anti-p53 mAbs, or fragments thereof. The different mAbs or fragments thereof may be administered concomitantly or sequentially at different intervals.

[0054] It is further encompassed by the present invention to administer to a patient effective amounts of two or more different peptides based on the same or different CDR regions of the same anti-p53 mAb or of different anti-p53 mAbs. The different peptides may be administered concomitantly or sequentially at different intervals.

[0055] Having now generally described the invention, the same will be more readily understood through reference to the following examples.

EXAMPLES

Materials and Methods

(i) Mice

(a) Female mice of the BALB/c strain were obtained from Olac, Oxon, UK, and used at the age of 8-10 weeks.

(b) Female mice of the inbred strain C57BL/6 were used at the age of 6-10 weeks. The mice were bred and kept at the animal breeding facilities of the Weizmann Institute of Science, Rehovot.

(ii) Monoclonal Antibodies (MAb)

[0056] The following mouse monoclonal antibodies (all IgGl/κ,) were used: mAb 240, specific for a mutated non-DNA binding conformation of the p53 molecule (Gannon et al., 1990); mAb 246, specific for the native conformation of p53 at the edge of the p53-specific DNA binding site (Cook and Milner, 1990; Yewdell et al., 1986); mAb 421, specific for the carboxy domain of p53 (Aral et al., 1986) and the anti-myconco-protein antibody mAb 9E10 (Munro and Pelham, 1986), used as a control antibody. R73 is a mouse-anti rat-T cell receptor monoclonal antibody (IgGl) (Hunig et al., 1989) and was used as a control antibody.

[0057] The antibodies were obtained by growing the hybridomas as ascites in BALB/c mice, collecting the ascitic fluids and purifying the antibodies by protein A affinity chromatography followed by dialysis in PBS.

(iii) Tumor Cells

(a) The Meth A tumor is a transplantable 3-methylcholantrene-induced sarcoma of BALB/c origin (Old et al., 1962) that has three mutations in the p53 coding sequence (Arai et al., 1986). The cells were grown in DMEM containing 10% heat-inactivated fetal calf serum (FCS), and passaged through BALB/c mice to select for tumorogenicity. For challenge, mice were injected intradermally with 10^6 Meth A

cells. Tumor growth was observed for eight weeks. Rejection was scored as the disappearance of the tumor mass within 20 days after tumor challenge.

(b) The 3LL-tumor (Eisenbach et al., 1983) is a metastasing lung, carcinoma cell line of C57BL/6 origin, that constitutively overexpresses p53, mostly wild-type. The cells were grown in DMEM containing 10% heat-inactivated fetal calf serum (FCS).

(iv) Immunization

[0058] Mice were immunized intradermally in the hind footpads with 20 µg of mAb in PBS in a 100 µl emulsion 1:1 in Complete Freund's Adjuvant (CFA; Difco Laboratories, Detroit, MI). An intradermal booster injection with 20 µg of the mAb in PBS alone was given three weeks later. Mice were bled at different times after the boost, and the sera were tested for specific antibodies.

(v) Recombinant p53 Proteins

[0059] E Coli BL21 (DE3) cells transformed with the T7 expression vector containing regularly spliced mouse p53 cDNA were used to express wild type or mutant p53 as previously described (Shohat-Foord et at., 1991; Wolkowicz et al., 1995). For protein induction, IPTG at a final concentration of 0.4 mM was added when the cells had reached an $O.D._{600}$ of 0.8-1.0. Three hours after induction, the cells were harvested from a

200 ml culture by centrifugation, and the pellet was
resuspended in 5 ml of ice-cold lysis buffer (20mM Tris-HCl,
pH: 7.4; 500 mM NaCl; 10% glycerol; 1 mM EDTA; 1 mM PMSF; 5
µg/ml leupeptine; 10 µg/ml aprotinin and 0.1% NP40). The

5   lysate was subjected to sonication for 10 cycles of 15 s of
sonication followed by 15 s of gentle swirling. The solicited
sample was subjected to two cycles of freeze-thaw and then
centrifuged for 10 min at 10.000 x g. The supernatant was
diluted with an equal volume of ice-cold water and an equal

10   volume of saturated ammonium sulfate was added drop-wise to
obtain a final concentration of 33% saturated ammonium
sulfate. After 15 min on ice and centrifugation, the pellet
was resuspended in 1 ml of lysis buffer and centrifuged for 5
min at 4°C. The supernatant was diluted with 4 ml of lysis

15   buffer devoid of NaCl. Wild type p53 and mutant p53 were
translated *in vitro* using Bluescript plasmids carrying the
specific cDNA (Promega, Madison, WI).

(vi) Antibody Assays

Antibodies to DNA or to p53 were detected using three

20   different assay systems. (i) immunoprecipitation was used to
detect antibodies to wild-type and mutant p53, as described
(Wolkowicz et al., 1995; (ii) gel-retardation was used to
detect antibodies to the synthetic oligonucleotide consensus
sequence specific for p53 (Wolkowicz et al., 1995); and (iv)

ELISA was done according to Waisman et al., 1993, using anti-mouse IgG and IgG1, IgG2a and IgG2b specific reagents to detect IgG antibody isotypes (Elias et al., 1997).

(vii) Cloning and Sequencing of Immunoglobulin H and L Chains

[0060] The polymerase chain reaction (PCR) was used to amplify mAb cDNA as described (Sambrook et al., 1989). Briefly, forward primers located in the constant region or in the J region and backward primers located in the V region, were used for amplification using Taq DNA polymerase s (Promega, Madison, WI, USA). The primers used for the PCR were described by Orlandi et al., 1989.

[0061] The PCR product was cloned, and ligated DNA was inserted into competent bacterial cells. Positive colonies were picked using specific internal primers that were labeled with g-ATP. Positive clones were grown and ssDNA was prepared as described (Sambrook et al., 1989). The ssDNA was sequenced using the Sequenase 2.0 kit (USB).

[0062] PCR products were separated from the primers used for the reaction using Primer Remover (Advanced Genetic Technologies, MD, USA). Around 1200 ng DNA were used for each sequencing (Applied Biosystems). Sequences were analyzed using Applied Biosystems and GCG package programs.

(viii) Peptide Synthesis

[0063] Peptides were synthesized as described (Konen-Waisman et al., 1995). Briefly, peptides were prepared with an automated multiple peptide synthesizer (Abimed Model AMS 422; Langenfeld, Germany) using the company's protocols for N-α-fluorenylmethoxycarbonyl(Fmoc) synthesis, or were prepared manually by a standard solid phase method (Merrifield, 1963) employing either N-α-Fmoc or N-α-t-butyloxycarbonyl (t-Boc) strategies. Crude products were purified by reversed phase HPLC on a semi-preparative C8-column (Lichrosorb RP-8, 7 µm, 250x10 mm, Merck, Darmstadt, Germany). Elution of peptides was achieved by linear gradients established between 0.1% trifluoroacetic acid (TFA) in water and 0.1% TFA in 75% acetonitrile in 25 water (v/v). The purity of the single peptide products was ascertained by analytical reversed-phase HPLC and amino acid analysis.

EXAMPLE 1: Induction of Anti-p53 Immunity

[0064] To exploit the idiotypic network to induce immunity to the p53 protein, BALB/cmice were unimmunized or were immunized with control mAb 9E10, or with anti-mutant p53 mAb 240 or anti-native p53 mAb 246. Ten days after the boost, the mice were bled and the sera were tested for antibodies to native p53 or mutated p53 by an ELISA assay, or by a

precipitation assay. Antibodies were measured after booster immurdzation.

[0065] For the ELISA assay flat bottom maxi-sorb plates (Nunc, Roskilde, Denmark) were coated with 50 µl per well of mutant or wild type p53 at a concentration of 10 µg/ml. After incubation with antigen, the plates were washed and blocked over night with 5% fetal calf serum (FCS; BioLab, Jerusalem, Israel) or with 1% bovine serum albumin (BSA; Sigma) in PBS. Test sera diluted serially (1/50 to 1/800) were then added for 90 min, followed by incubation for 75 min with 50 µl per well of alkaline phosphatase-conjugated goat anti-mouse IgG, F_c fragment (Jackson Immunoresearch Laboratories, Inc. West Grove, PA). After washing, the plates were incubated with the substrate, p-nitrophenil phosphate disodium (Sigma) and read using an ELISA reader at 405 nm. Sera manifesting an O.D. reading, after substraction of the background, of 0.3 or greater, were scored as positive.

[0066] For the immunoprecipitation assay, TCA-insoluble fraction of recombinant wild type or mutant p53 or lysates of Meth A tumor cells known to produce mutant p53 (Arai et al., 1986) metabolically labeled with ^{35}S-methionine, were reacted with antibodies or test sera for 2 h at 4°C. The immune complexes were precipitated with Sepharose Protein A, separated on SDS-PAGE, electrotransferred to nitrocellulose

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membranes, and the proteins were detected using the Protoblot western blot Ap system (Promega, Madison, WI).

[0067] The results, summarized in Table 1, show that unimmunized control mice and mice immunized with control mAb 9E10 were not positive for anti-p53 antibodies. In contrast, mice immunized with anti-mutated p53 mAb 240 showed antibodies to both the mutated and native forms of p53. Mice immunized with anti-wild type p53 mAb 246 were positive for anti-p53 antibodies to wild type p53 but not to mutated p53. Analysis of the isotypes of the anti-p53 antibodies using the ELISA assay indicated that the antibodies included those of the IgG2a isotype regulated by T-helper 1 type cells that secrete interferon gamma (IFN-γ; Elias et al., 1997).

Table 1
Mice Immunized with anti-p53 mAb Develop Immune Reponses to
p53

Immunogen	Antibody Assay	
	Antigen	Incidence of Positive Mice
None	p53	0/20
	mutated 53	0/20
mAb 9E10	p53	0/20
	mutated p53	0/20
mAb 240	p53	10/10*
	mutated p53	10/10*
mAb 246	p53	10/10*
	mutated p53	0/10
mAb 421	P53	10/10*
	mutated p53	10/10*

* p < 0.01

[0068] Fig. 1 shows the retardation in gel of the p53-specific oligonucleotide sequence by anti-DNA antibodies developing only in the mice immunized with mAb 246, but not

5   with mAb 240, mAb 421, mAb 9E10 or mAb R73. This result indicates that anti-DNA antibodies may recognize specific sequences of DNA.

[0069] Fig. 2 shows a precipitation assay using an extract of the Meth A tumor metabolically labeled with S^{35}. This tumor

10   produces mutant p53. It can be seen that mAb 240 (anti-mutant

p53) precipitated the mutant p53 band, while mAb 246 did not precipitate the mutant p53 band, but did precipitate wild-type p53 (not shown). The mice immunized with mAb 421 or with mAb 240 developed specific anti-p53 antibodies. The mice

5 immunized with mAb 246 or with mAb 9E10 did not develop antibodies to mutant p53. Immunoprecipitation of recombinant p53 confirmed these results.

EXAMPLE 2: Immunized Mice Reject Tumors

[0070] Antibodies to tumor antigens have not been found to

10 be effective in rejecting solid tumors (LoBuglio and Saleh, 1992; Mellstedt et al., 1991; Velders et al., 1995). To learn whether immunization with anti-p53 mAb might influence *in vivo* the development of a tumor bearing a mutated form of the p53 molecule, groups of BALB/c female mice were immunized as above

15 with either mAb 240, mAb 246, mAb 421 or mAb 9E 10. The mice were challenged intradermally with 10^6 Meth A tumor cells 10 days after the boost. Non-immunized mice were also tested. The results in Table 2 are expressed as the incidence of the mice that rejected the tumor in comparison to the mice that

20 did not reject the tumor.

Table 2
Immunization with anti-p53 mAbs activates rejection of
Meth A Sarcoma Cells

Immunogen	Incidence of Rejected Tumors
None	0/30
mAb 240	9/10*
mAb 246	9/10*
mAb 421	9/10*
mAb9E10	0/10

* P < 0.01 compared to non-immunized control mice (none)

[0071] Table 3 summarizes the antibody results and also shows that the mice that anti-p53 antibodies also rejected the Meth A tumor. The tumor rejection experiments have been repeated 4 times and have involved 30-40 mice per group. About 90% of the mice developing p53 immunity rejected their tumors. It is quite possible that T cells were involved in the rejection process. However, it has been shown that intact antibodies can enter living cells (Alarcon-Segovia et al., 1996; Madaio et al., 1996; Yanase et al., 1994; Ma et, al., 1991) and it is conceivable that the anti-p53) antibodies themselves killed the tumor cells, possibly by inducing apoptosis (Madaio et al., 1996). It is also possible that the anti-p53 mAbs exerted an anti-tumor effect through antibody-dependent cytotoxicity (ADCC). The antibodies may also have acted in synergy with T cells (see Vasovic et al.,1997).

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[0072] The growth of the Meth A tumor cells was inhibited in mice immunized with mAb 240, mAb 421 or mAb 246. In contrast to the protection induced by these mAbs, no protection was induced by immunization to control mAb 9E10. Thus, resistance to tumorchallenge was associated with the anti-p53 mAbs.

[0073] It can thus be summarized from the above results that immunization with anti-p53 mAb molecules induces anti-p53 immunity and can induce anti-DNA antibodies. The anti-DNA antibodies can show specificity for DNA recognized by different p53 domains. The anti-p53 autoimmunity is associated with tumor rejection. These results are summarized in Table 3.

Table 3
Immunization to Anti-p53 mAb Molecules Induces Specific Anti-DNA and Anti-p53 Immunity. The Anti-p53 Immunity Is Associated with Tumor Rejection

		Induced Reactivities		
mAb	Specificity	Anti-DNA Specific for the p53 Reactive Sequence	anti-p53	Tumor Rejection
9E10	Myc	No	No	No
240	mutated p53	No	Yes	Yes
246	p53-specific domain	Yes	Yes	Yes
421	p53-carboxy domain	No	Yes	Yes

[0074] Although antibodies naturally arising in tumor patients to the amino-terminal domain of p53) are not protective and may even indicate a poor prognosis (Schlichtholz et al., 1992), the rejection of tumors by mAb treatment may be explained by the fact that the mAbs were specific for other domains of p53. Moreover, the generation of IgG antibodies by the mAb treatment suggests that T cells may be involved. Be that as it may, the induction of anti-p53 reactivity by the use of specific mAbs is functionally effective.

EXAMPLE 3: Preparation of CDR-Based Peptides of Anti-P53 mAbs

[0075] The light (L) and heavy (H) chains of the anti-p53) mAbs 240, 246, 248 and 421 were cloned and sequenced as described in Materials and Methods, section vii. The sequences are shown in Fig. 3 (CDR sequences are underlined).

[0076] Peptides V-IX described hereinabove were synthesized as described in Materials and Methods, section viii.

EXAMPLE 4: CDR-Based Peptides of Anti-p53 mAbs Induce Anti-p53 Immunity in Mice

[0077] Immunization of BALB/c mice with peptides V-IX of Example 3 was carried out as described for the whole mAbs in Materials and Methods, section iv, using 100 µg of peptide, and then assayed for the development of anti-p53 antibodies.

[0078] The incidence of mice developing IgG anti-p53 antibodies (ELISA assay) was 8/10 and 7/10 for peptides V and VI, the CDR3-based peptides of mAb 240 and mAb 421, respectively.

[0079] The results of another experiment carried out with peptides V-IX (Table 4) show that mice immunized with these peptides rejected the Meth A tumor.

Table 4
Rejection of Meth A Tumor in Mice Immunized with
Anti-p53 mAb CDR-Based Peptides V-IX

Peptide Immunogen	Incidence of Rejected Tumors
None	0/5
V	4/5
VI	5/5
VII	5/5
VIII	4/5
IX	4/5

[0080] These experiments show that fragments of anti-p53 mAb, e.g. CDR-based peptides, can be used to induce anti-p53 immunity.

EXAMPLE 5: Prevention of Lung Carcinoma Metastasis by Immunization with Monoclonal Antibodies to p53

[0081] Since the most likely application for immunotherapy of tumor is not only the treatment of solid tumors, but rather

the treatment of metastasis after surgical removal of a solid

tumor, the anti-p53 mAbs were also applied in a model of a

metastasing tumor.

[0082] Immunization of C57BL/6 mice was performed with

anti-p53 mAbs 240, 246, 421 and mAb R73 as control, as

described in Materials and Methods, section (iv). Ten days

after the boost, the mice were injected intradermally in the

hind footpads with $2x10^5$ 3LL-lung carcinoma cells. Tumor

growth at the injection site was observed. The tumors were

excised when they reached 8 mm. The mice were sacrificed 21

days after tumor removal and their lungs were weighed as a

quantitative measure of the metastatic load. In case of

spontaneous mortality, the lung weight was determined on the

day of death. Fig. 4 shows the protective effect of the p53

antibodies against the development of lung metastasis. All

three anti-p53 mAbs tested significantly protected the 3LL-

challenged mice against metastasis ($p < 0.01$, when compared to

R73).

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